HEAVY NICOTINE AND ALCOHOL USE IN ALCOHOL DEPENDENCE IS ASSOCIATED WITH D2 DOPAMINE RECEPTOR (DRD2) POLYMORPHISM

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Abstract

Cigarette smoking in those who are alcohol dependent is associated with higher morbidity and mortality. The A1 allele of the D_2 dopamine receptor (DRD2) gene has been independently associated with alcohol and nicotine dependence. Whether this polymorphism is associated with nicotine dependence in those who are also alcohol dependent has not been investigated. Subjects were 84 (61 males; 23 females) Caucasian DSM IV diagnosed nicotine-and alcohol-dependent subjects sampled from consecutive admissions to a hospital alcohol detoxification ward. Data were obtained through standardised measures of nicotine and alcohol consumption and dependence severity. $A1^+$ allelic (A1/A1 or A1/A2 genotype) compared to $A1^-$ allelic (A2/A2 genotype only) patients were characterised by higher levels of alcohol and cigarette consumption. $A1^+$ allelic patients reported greater alcohol dependence severity, but not nicotine dependence severity. When the combined nicotine and alcohol dose was examined, $A1^+$ allelic patients consumed significantly more of these drugs than their $A1^-$ allelic counterparts.

1. Introduction

The World Health Organization has listed tobacco and alcohol misuse as being among the five most significant risk factors associated with the global burden of disease (Powles & Day, 2002). Tobacco smoking will become the leading global disease burden by 2020 (Murray & Lopez, 1996). There is a significant association between alcohol and tobacco misuse (Bein & Burge, 1990), with high levels (>80%) of comorbid dependence (Pomerleau, Aubin & Pomerleau, 1997). While, the morbidity and mortality attributed to these substances alone is considerable, the combined effects of cigarette smoking and heavy alcohol consumption represent an even higher disease risk. Recent meta-analyses show that concurrent tobacco and alcohol misuse significantly increase the risk for upper digestive tract (Bagnardi, Blangairdo, La Vecchia & Corrao, 2001) and respiratory tract (Korte, Brennan, Henley & Boffetta, 2002) carcinoma. Up to one-third of oral cavity cancer risk has been attributed to the combination of cigarette smoking and alcohol consumption amongst males (Balaram et al., 2002). Furthermore, there is a combined influence of cigarettes and alcohol on respiratory disease. Alcohol consumption itself is a significant risk factor associated with lung capacity decline (Burchfiel et al., 1996; Ström et al., 1996). A large scale German epidemiological study of over seven million hospital admissions found that 32.2% of the total in-patient treatment costs were associated with tobacco- or alcohol-attributed disease (John, Rumpf, Hanke, Gerke & Hapke, 2003).

The negative impact of cigarette smoking on health in those with an alcohol problem is illustrated by retrospective data examining the cause of death of 845 alcohol-dependent individuals (Hurt et al., 1996). These data showed that amongst those with alcohol

dependence, the effects of alcohol were responsible for one-third of deaths, whereas smoking accounted for more than half of the deaths. In terms of psychosocial morbidity, the quality of life and mental health of those with alcohol problems (Connor, Saunders & Feeney, 2006; Foster, Powell, Marshall & Peters, 1999) and smokers (Olufade et al., 1999) is rated poorly.

The development of comorbid nicotine and alcohol dependence is influenced by a complex interplay of genetic and environmental factors. Twin studies have demonstrated the heritability component of alcohol (Pickins et al., 1991; Prescott, Aggen & Kendler,1999) and nicotine (True et al., 1997) misuse to be in the order of 60%. It is likely that much of this genetic variance is shared (Koopmans, van Dooren & Boomsma, 2002). Association studies which compare the frequency of candidate genes between drug-dependent and non-drug-dependent groups have proposed a range of potential genetic markers for alcohol and nicotine dependence (Fergusson & Goldberg, 1997). One such marker, the D_2 dopamine receptor (DRD2) gene, has shown considerable promise across independent association studies of substance dependence, including alcohol and nicotine. For example, in an analysis of the Collaborative Study on the Genetics of Alcoholism (COGA) data, an independent research group found that only two regions, one containing the candidate gene ADH3, the other the DRD2, were implicated in alcohol dependence (Waldman, Robinson & Rhee, 1999). While the majority of association study meta-analyses have consistently found a higher prevalence of the DRD2 A1 allele in individuals with alcohol misuse disorders compared to controls (Cloninger, 1991; Gorwood, Ades & Feingold, 1994; Gurling & Cook, 1999; Lawford et al., 1997; Noble, 2003; Pato, Macciareli, Pato, Verga & Kennedy, 1994;

Young et al, 2004) one meta-analysis (Gelernter, Goldman & Risch,1993) has not (see Noble & Blum, 1993). The increasing frequency of $A1^+$ allelic status (A1/A1 and A1/A2 genotypes) is associated with increasing severity of alcohol dependence (Lawford et al., 1997; Noble, 2003). This is evident in multiple ethnic or racial groups suggesting that the association described is not simply due to population stratification (Young et al, 2004).

A recent meta analysis of candidate genes potentially related to smoking confirmed the association between the A1 allele of the DRD2 and smoking initiation as well as response to treatment (Munafo et al., 2004). $A1^+$ allelic status is more likely found in smokers than non-smokers (Noble et al., 1994; Comings et al., 1996; Pastorelli et al., 2001), although Singleton et al., (1998), using a combined community and post-mortem sample, found no association between the DRD2 A1 allele and smoking status. However, a meta-analysis of 9 different studies of 1,721 smokers and 2,119 non-smokers (Noble, 2003) found the prevalence of the A1 allele to be significantly higher in smokers than those who do not smoke ($p = 1.29 \times 10^{-7}$). The A1 allele is also associated with number of cigarettes smoked (Waldman, et al., 1999) and the familial aggregation of smoking-related cancers (Wu, Hundmon, Detry, Chamberlaine & Spitz, 2000). Further, DRD2 $A1^-$ (A2/A2 genotype only) allelic status is also associated with a greater likelihood of never having smoked (Anokhin, Todorov, Madden, Grant & Heath, 1999; Lerman et al., 1999). Moreover, the A1+ (A1A1 or A1A2 genotype) allelic status is more common amongst polysubstance misusers (Smith, et. al., 1992), although the subgroup of heavy drinkers and cigarette smokers were not examined separately.

In sum, the DRD2 A1 allele is individually associated with severe alcohol and nicotine misuse problems. Whether this polymorphism is associated with nicotine misuse in those who are also alcohol dependent has not been investigated. This study aims to explore this relationship by examining DRD2 allelic status and key drinking and smoking indices within an alcohol-dependent population.

2. Methods

2.1 Participants

Subjects in this study were 84 Caucasian inpatient smokers (61 males; 23 females) sampled from consecutive admissions to a hospital alcohol detoxification ward. These smokers were recruited from a larger study investigation alcohol treatment issues (Connor et al., 2002).

Subjects were recruited from days 2-5 of their detoxification. All patients (a) satisfied DSM-IV criteria for Alcohol and Nicotine Dependence. Nicotine Dependence diagnosis was confirmed by exceeding the nicotine-dependent cut off on the Fagerstrom Tolerance Questionnaire (Fagerstrom, 1978), (b) were unrelated Caucasians, (c) were not current polysubstance or illicit drug misusers (with the exception of greater than once per month marijuana misuse), (d) did not have any life-threatening alcohol-related medical complications, (e) were physically and mentally well enough to undertake a clinical interview, and (f) provided written informed consent to participate in the clinical interview

and to have blood drawn for DNA analysis. The mean age of these 84 subjects was 40 years (*s.d.* = 9.25 years, *range* = 22-63 years).

2.2 Clinical Assessment

Medical staff initially assessed all patients and a psychologist subsequently conducted the clinical interview. The interview included basic demographic information and assessed tobacco/alcohol consumption and severity of nicotine/alcohol dependence. The research protocol was approved by the Ethics Committee of the Royal Brisbane and Women's Hospital and The University of Queensland. All patients provided informed written consent to participate in the study.

2.2.1 Consumption: Nicotine

Cigarettes smoked per day was assessed by the question: "On average, how many cigarettes do you smoke each day?" The clinician assisted the patient with quantification, for example to assess if there was a variation between weekday smoking and smoking at weekends. If the patient made his or her own cigarettes rather than smoked manufactured cigarettes, the clinician asked the patient to show them the typical thickness of the cigarette. A transformation to standard manufactured cigarettes was then made. Smoking frequency data were not examined separately as all smokers recruited reported smoking on a daily basis.

Nicotine content of cigarettes in milligrams was assessed by asking the patient to report the "strength" of cigarettes smoked (e.g. 0.2 mg, 0.4mg, 0.8mg, 1.6mg of nicotine). If the

nicotine was assessed by content was unknown, the subject's cigarette packet was examined or the brand noted so this could be established following the assessment.

Nicotine dose consumed per week was determined by the composite variable consisting of Average Cigarettes Smoked per Day x mg content x 7.

2.2.2 Consumption: Alcohol

To fully describe alcohol consumption, both quantity and frequency measures were obtained (Rehm, 1998) using previously validated measures (Connor, Young, Williams & Ricciardelli, 2000; Connor, Gudgeon, Young, & Saunders, J.B., 2004; Gudgeon, Connor, Young & Saunders, 2005). The recommendations from the Skarpo conference (Dawson & Room, 2000) were followed, in that (i) Quantity of drinking measurement captured information in terms of quantity of drinks per occasion rather than quantity of drinks consumed per day and, (ii) Frequency of drinking should not be asked in an open-ended manner and should have a specified range in terms of times per week.

Alcohol Quantity was assessed by the question: "On average, how many drinks do you consume on each daily drinking occasion?" Subjects were first shown pictures of a range of common alcohol servings (glasses, bottles, etc.), which included volume from colloquial Australian expressions ("pots", "midis", "schooners", etc.). This was then transformed to a standardised measure (ie. grams of absolute alcohol).

Drinking Frequency was the average number of drinking days per week (*range*= 0 to 7).

Ethanol Dose Per Week was calculated by multiplying Alcohol Quantity by Drinking Frequency.

2.2.3 Dependence Severity

Nicotine: The degree of physical dependence on nicotine was assessed by the Fagerstrom Tolerance Questionnaire (FTQ, Fagerstrom, 1978). FTQ scores correlate well with physiological measures of nicotine use such as cotinine level (Fagerstrom & Schneider, 1989; Pomerleau, Pomerleau, Majchrazak, Kloska & Mulakuti, 1990).

Alcohol: The Alcohol Dependence Scale (ADS, Skinner & Allen, 1982, Skinner & Horn, 1984) questionnaire was used to quantify alcohol dependence severity. The ADS is a reliable and valid instrument for assessing level of dependence (Skinner & Allen, 1982).

2.3 Genotyping

A 10 ml blood sample was drawn from each patient. Genomic DNA was extracted employing standard techniques and used as a template for determination of *Taq*I A DRD2 alleles by the polymerase chain reaction (Grandy, Zhang & Civelli 1993). The amplification of DNA was carried out using a Perkin Elmer GeneAmp 9600 thermocycler. Approximately 500 ng of amplified DNA was digested with 5 units of *Taq*I restriction enzyme (New England Biolabs) at 65°C. The resulting products were separated by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualized under ultraviolet light. The A1/A2 genotype is revealed by three fragments:

310 bp, 180 bp and 130 bp; the A2/A2 genotype by two fragments: 180 bp and 130 bp; and the A1/A1genotype is shown by the uncleaved 310 bp fragment. All genotyping was carried out blind to the subjects' results in the clinical assessment in that the DNA data were not available to the researchers until the clinical assessments were completed. $A1^+$ allelic subjects were those that either had the A1/A1 or A1/A2 genotype; $A1^-$ allelic subjects had the A2/A2 genotype only.

2.4 Statistics

Analyses on the interval data were conducted by multivariate and univariate Analyses of Variance (ANOVA). Analyses on the nominal data were conducted by chi square statistic (Fisher's Exact Test). All analyses were conducted with SPSS Version 11. A *p* value of # .05 was considered to be statistically significant.

3. Results

3.1 Genotypes

Of the 84 smokers in this study, 30 had the $A1^+$ allele (four A1/A1 and 26 A1/A2 genotypes) and 54 had the $A1^-$ allele (A2/A2 genotype). A1 allelic frequency = 0.20 and A2 allelic frequency = 0.80. In the excluded sample of non-smokers, 5 subjects had the $A1^+$ allele (one A1/A1 and four A1/A2 genotypes) and 17 had the $A1^-$ allele (A2/A2 genotype). A1 allelic frequency = 0.14 and A2 allelic frequency = 0.86. There was no significant difference in allelic frequencies between smokers and the excluded non-smokers ($\chi^2 = 0.61$, $p = .44$).

3.2 Sex and Age Variables

To investigate possible variation in consumption on the basis of sex and age proportions between allelic groups, two chi-square analyses were undertaken. There was no significant difference in the proportion of males and females in either allelic group (χ^2 = 2.70, p= 0.13). Similarly, there was no significant difference in mean age (at assessment) between allelic groups. The mean age for $A1^+$ allelic subjects was 39.9 years (s.d. = 10.6) and 40.0 years (*s.d.* = 8.5) for $A1^-$ allele subjects (F (1, 82) = 0.005, p = .94).

3.3 Smoking and Drinking Variables by Allelic Group

To investigate if there was an overall difference in allelic status between smoking and drinking variables, a MANOVA was conducted on the 84 smoking subjects. Composite variables (i.e. weekly mg nicotine consumption and weekly standard drinks consumed) were considered separately, as they possessed a high level of multicollinearity (Hair, et al., 1995). An overall allele effect trend was found (Hotelling's T^2 = .858, F [6, 77] = 2.12, p =.06). There was a significant difference between allelic groups within the composite smoking and drinking variables (Hotelling's T^2 = .150, F [2, 81] = 6.08, p =.003), with the $A1^+$ allelic participants reporting a higher consumption than their $A1^-$ allelic counterparts. Table 1 provides the effect size, means and standard deviations of the univariate analyses for all smoking and drinking variables.

INSERT TABLE 1 HERE

3.4 Smoking and Drinking Severity: Standard Score Composite by Allele Group

To examine the relationship between allelic status and the combined effect of smoking and drinking, Total Nicotine Dose per Week and Ethanol Dose per Week per were initially transformed to standard (*Z*) scores. The range for Total Nicotine Dose per Week was -1.46 to 2.06 and -1.56 to 4.71 for Ethanol Dose per Week. The two standard scores were combined to form a composite nicotine/alcohol dose per week. ANOVA indicated that that the $A1^+$ allelic group reported a significantly higher combined nicotine/alcohol dose (*mean*= .82, *s.d.* = 1.78) than the $A1^-$ allelic subjects (*mean*= -.37, *s.d.* = 1.37) (F (1, 82) = 11.68, p = .001).

To further examine the hypothesis that individuals carrying the $A1^+$ allele are likely to consume higher substance doses of both nicotine and alcohol than subjects with the $A1^-$ allele, a median split procedure categorised the sample into low/high Nicotine Dose per Week and low/high Standard Drinks per Week. Subjects were then allocated to four categories a) Low Nicotine per Week/Low Alcohol per Week (*n*=23), b) Low Nicotine per Week/High Alcohol per Week (*n*= 15), c) High Nicotine per Week/Low Alcohol per Week (*n*=17), d) High Nicotine Per Week/High Alcohol per Week (*n*=29). To examine whether the A1 allele was a marker of severity in the current sample the three groups representing heavy use of either alcohol or nicotine or both (Groups b, c and d) were

combined to constitute a severe use sample and compared to the group that used both substances at below the median (Group a). Chi-square analysis indicated a significant difference in allelic status between the severe misuse sample and those using both cigarettes and alcohol below the median ($\chi^2 = 4.63$, $p=.03$).

4. Discussion

The current study reports the association of the A1 allele of the DRD2 with severity markers of substance misuse amongst problem drinkers who are also nicotine dependent. $A1^+$ allelic participants were exposed to greater health risk via their combined consumption of a higher number of cigarettes and a higher dose of alcohol than their $A1^-$ allelic counterparts. Prior studies have shown independent associations of DRD2 allelic status with smoking and drinking, however the examination of combined risk has not previously been assessed. It is well-documented that individuals that are nicotine-dependent have a greater risk of alcohol dependence severity (Daeppen et al., 2000). The carcinogenic risk (particularly of the oropharyngeal region and gastrointestinal tract) associated with the combination of heavy cigarette and alcohol use is enhanced. The A1 allele is associated with a familial aggregation of smoking-related cancers in research where smoking behaviour was measured but the role of alcohol was not examined (Wu et al, 2000).

These findings underscore the importance of establishing a comprehensive substance use history in association studies of the A1 allele of the DRD2. Given that the DRD2 A1

allele has also been associated with severe substance misuse across a range of substances (for a review see Young, Lawford, Nutting & Noble, 2004a), it is clearly not specific for alcohol or nicotine dependence. It is more likely to be associated with neurochemical reward mechanisms (Noble, 1996; Young, Lawford, Feeney, Ritchie & Noble, 2004b). Previous research has emphasised the importance of the DRD2 as a general risk factor for substance misuse (Noble, 2003; Lawford et al, 1997; Young et al, 2004a) rather than a marker of risk for a particular drug. As multiple substances influence dopaminergic activity, the examination of a single substance may result in an incomplete examination of allelic risk. This study indicates that amongst those seeking treatment for alcohol-dependence who are also smokers, the subgroup using the most nicotine or alcohol, either alone or in combination, were more likely to carry the A1 allele.

Given the common risk for both severe alcohol and/or nicotine misuse associated with the A1 allele, it is important that the potential to treat both disorders in a manner that heeds this risk is investigated. Those with alcohol-dependence have been shown to be receptive to undertake treatment of both disorders simultaneously (Saxon, McGuffin & Walker, 1997). Previous pharmacogenetic treatment of alcohol dependence using bromocriptine, a dopamine agonist that acts at the D2 dopamine receptor has shown an interaction between DRD2 allelic and medication status with reductions in craving only being evident in the $A1^+$ allelic group treated with bromocriptine (Lawford et. al., 1995). Recent research indicates that bromocriptine has promise in smoking cessation (Murphy et al., 2002) and future research should examine the addition of bromocriptine, or a similar D2 dopamine receptor agonist, to current treatments to facilitate the pharmacogenetic treatment of these

comorbid disorders.

The current study is limited by the severe nature of the substance misuse problems within the sample and may not generalise to those who are not in treatment settings. Furthermore, the data are cross-sectional and are based on self report alone, without biochemical verification. In addition, examination of other aspects of smoking behaviour including craving, withdrawal severity and subjective reinforcement of nicotine may help to further elucidate the impact of the A1 allele.

In summary, DRD2 $A1^+$ allelic status is associated with increased misuse of alcohol and nicotine and the heavy comorbid misuse of both substances in an alcohol-dependent sample. The evidence supports the association of the A1 allele with severe substance misuse. This common genetic association of alcohol and nicotine dependence allows for the possibility of a targeted pharmacogenetic approach that may ameliorate both disorders simultaneously producing improved general health and decreased risk for associated upper digestive and respiratory tract disorders.

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Table 1. Smoking and drinking parameters by DRD2 allelic status*

Measure	A_1+ Allele		A_1- Allele		Effect Size	
	Mean	*S.D.*	*Mean*	*S.D.*	*F*	*P*
Smoking Indices						
Cigarettes per day	36.2	16.8	27.5	14.3	6.34	.014
Nicotine content (mg)	1.36	.41	1.17	.47	3.55	.063
Fagerstrom Tolerance Questionnaire	7.07	2.61	6.24	2.83	1.74	.191
Drinking Indices						
Drinking Frequency (days per week)	6.20	1.58	6.06	1.37	.19	.662
Drinking Quantity per day (g)	217	106	167	84	5.70	.019
Alcohol Dependence Scale (ADS)	33.76	9.34	29.94	8.89	3.44	.067
Composite Variables						
Nicotine dose per week (mg)	364	203	235	168	9.81	.002
Ethanol dose per week (g)	1366	768	1031	587	5.00	.028

* A_1^+ allele are individuals with the A1A1 or A1A2 genotype, A_1^- allele are individuals with the A2A2 genotype only.